A STUDY OF LABORATORY METHODS TO DETERMINE STRAWBERRY VARIETAL RESISTANCE TO GREY MOLD (BOTRYTIS CINEREA)

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Thomas Bruce Irvine

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A STUDY OF LABORATORY METHODS TO DETERMINE STRAWBERRY VARIETAL RESISTANCE TO GREY MOLD (*BOTRYTIS CINEREA*)

By

Thomas Bruce Irvine

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ABSTRACT

An investigation of techniques that could be employed in a laboratory screening test to evaluate relative varietal fruit susceptibility to Grey Mold (Botrytis cinerea) was undertaken.

Strawberry juice was extracted and sterilised by Seitz filtration. These strawberry juices were incorporated in both liquid and solid media and evaluated for growth of Botrytis cinerea. Growth responses were consistent for certain strawberry varieties throughout these methods.

Comparing pH and percent soluble solids of the juices with the growth responses obtained in the petri plate, growth tube and shake culture methods of evaluation indicated with the exception of Z-2, which supported Botrytis under the petri plate method only, all varieties having a pH below 3.0 consistently failed to support Botrytis in all three methods. No correlation was indicated between results obtained and percent soluble solids, or varieties having a pH above 3.0.

Studies of spore germination using hanging drop preparations of juice directly extracted from berries and inoculated with Botrytis spores was undertaken. An attempt was made to determine the influence of maturity or stage or ripeness of the fruit and variety upon the growth of the germ tubes of B. cinerea.

Tests of correlation between results obtained in the germ tube length in the juice of ripe fruit and the mycelial penetration of whole ripe fruit gave a highly significant correlation coefficient. It is suggested that the spore germination method be used as a preliminary screening test in a breeding program followed by whole fruit penetration studies later in the program. Additional testing in subsequent seasons should disclose whether a definite pattern of response exists.

TABLE OF CONTENTS

INTRODUCTION

Grey Mold fruit rot, *Botrytis cinerea*, inflicts losses estimated at over $100,000 a year to the Michigan strawberry grower (6). Control of this fruit rot by fungicidal sprays has improved greatly within the past ten years, but spraying is costly and if improperly timed is ineffective.

The control of Grey Mold by varietal resistance is one of the ultimate goals of the strawberry breeder. Evaluation of varieties, selections and seedlings to Grey Mold resistance by field plot observations has been inconsistent in the past, partly due to the variability of the micro climate and the inoculum potential. Thus an investigation of techniques that could be employed in a laboratory screening test to evaluate relative varietal fruit susceptibility to Grey Mold fruit rot was prompted.

REVIEW OF LITERATURE

Grey Mold fruit rot, caused by Botrytis cinerea, was first described on strawberry by F. L. Stevens in 1914 (8). More than 100 different host plants have been reported for Botrytis cinerea (2). It may be found on all sorts of decaying vegetative matter and on the mulch and old leaves of the strawberry bed in any season of the year (1).

On strawberry, Grey Mold attacks flower bud pedicels, stems and calyces of small green fruit and leaf petioles (11,12) and it is not unusual to find an entire fruit cluster infected at once (1). Besides causing heavy loss in yield, such infection builds up a high inoculum potential which under conditions of high humidity and temperatures around 60°F (6) will invade the ripening crop with a heavy spore load impossible to control with sprays during harvest (12).

Botrytis cinerea is able to penetrate unwounded tissue (1). Brown (5) found that penetration of the cuticle must take place in a purely mechanical way, the infecting germ tubes being unable to affect chemically the cuticle of the host nor are they able to secrete any toxic substance which can pass through the cuticle or bring about death to the underlying cells. This pressure theory was backed by Blackman and Welsfords' (4) work on Vicia faba.

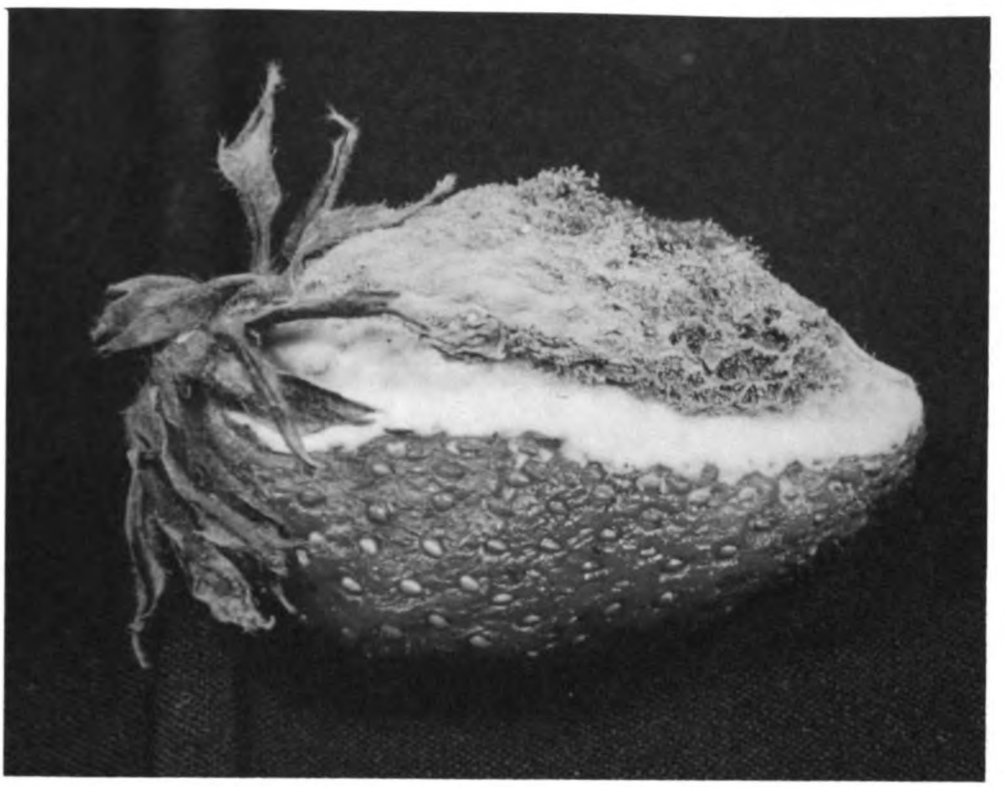

Figure I Botrytis cinerea, Grey Mold fruit rot, on the Premier variety. The advanced stage of this rot is typified by masses of grey conidia as noted on upper portion of berry.

Webb (14) investigated the effect of hydrogen ion concentration on germination of Botrytis conidia and found that variations in range occurred dependent upon the media. Germination occurred at a pH as low as 1.6 in a manite solution and as high as pH 10.0 in a beet decoction. In general the optimum pH for germination was in the area of 3.0. Tribe (13) found that enzyme preparations from culture filtrates were active from pH 3.5 to 6.0 and that activity decreased rapidly from 6.0 to 8.0.

Stevens (11) noted no difference in varietal susceptibility when an epidemic of Botrytis swept through an experimental plot containing over three hundred varieties. Anderson (1) also found no difference between varieties as regards susceptibility to infection by Botrytis. However he noted that habit of growth caused a striking difference in the amount of Grey Mold which developed in varieties. Varieties which have compact growth and long leaf stems which shade fruit are more likely to rot.

Within the fruit, the fungus is evidently capable of readily dissolving the middle lamella and of penetrating the cell walls themselves (9). Hyphae grow through the berry rotting the tissue (1) and the whole berry is rapidly involved, finally becoming covered with conidia (8). The affected berries retain their shape, begin to shrivel but no leakage of juice occurs (9).

The berries are even firmer than normal berries, some eventually becoming hard and dry (1,11).

Data of Miller and Waggoner (7) collected from a Hirst spore trap suggested that most infection by Botrytis cinerea originates from nearby primary inoculum and that the microclimate afforded by the dense foliage is more important than environmental conditions above the plant in determining incidence of Grey Mold.

Sclerotia are produced by the fungus as a protection against dry weather. These lodge in the mulch or drop to the ground and when moisture is present, send out vegetative hyphae which in turn produce conidia (1).

Epiphytotics of Grey Mold are associated with abundant moisture (1,3,17). Wilson (16) in studying the effects of irrigation found that on one occasion when very heavy rainfall followed heavy irrigation and another occasion when humid atmosphere prevailed in spite of a dry soil, the increase in crop was offset by an increase in rotten fruit and the marketable crop was slightly less or no more than that from unwatered plots.

In culture, growth of Botrytis cinerea at 0°C is sparse; at 2°C it grows freely; growth at 25°C is luxuriant and this temperature is considered in the optimum range for growth falls off sharply at 30°C (10).

Evidence of exploration of variation in the ability of the fruit of different varieties to support Grey Mold is apparently lacking in the literature.

MATERIALS AND METHODS

1. Preparation of Strawberry Juices

Ripe fruit samples of strawberry selections and varieties were taken at random from trial plots located at the C. O. Zollar Nursery, Benton Harbor, Michigan. A puree was then made from these berries by means of a laboratory puree machine, placed in erlenmeyer flasks, and stored at 10°C.

In order to facilitate juice extraction from the purees, one to two cups of moist canners cellulose was mixed with each liter of puree before pressing. The juice was then extracted using a hydraulic press at approximately 2500 pounds of pressure per square inch.

After extraction, the individual juices were passed first through a #C3 clarifying pad on a 60 mm Seitz filter using both vacuum and air pressure (Fig.2). This was followed by passage through a #ST-3 sterilizing pad and then final aseptic passage through another #ST-3 sterilizing pad.

2. Solid Media

a. Petri Plate Method

To quickly and accurately measure the liquid media and agar used in this and following methods, an automatic syringe was constructed. This consisted of a 20 ml hypodermic syringe inserted through a #13 rubber stopper. A six inch bolt inserted through a

Figure II Seitz filter and suction flask apparatus used to clarify and sterilize the strawberry juice.

#6 rubber stopper was used as an adjustable plunger stop. This bolt was run through the #13 stopper adjacent to the syringe and was held in place by nuts on both sides of the stopper. A two inch 18 g hypodermic needle completed the apparatus (Fig.3).

Botrytis cinerea was cultured at 20°C in petri dishes containing potato dextrose agar (Difco). Discs, 1.5 cm in diameter, were cut from the perimeter of the growing culture using a sterile pyrex test tube.

Seven ml portions of the sterile strawberry juice were aseptically pipetted to sterile 90 mm petri plates and mixed with seven ml of cool but fluid sterile 3% Difco agar. When solid, the samples were inoculated in the center with discs of Botrytis cinerea.

Two methods of inoculum placement were evaluated in this test. One in which the inoculum was placed upright and the other in which the inoculum was inverted on the agar juice preparation. It was observed that when the inoculum disc was inverted and became somewhat sealed with the agar, the mycelial growth was delayed slightly in comparison to placing the inoculum disc in an upright manner. The inoculum disc was placed upright on the agar juice preparation.

Inoculated samples were incubated at 20°C and growth measurements taken periodically. Measurements, were made in two directions at right angles, each

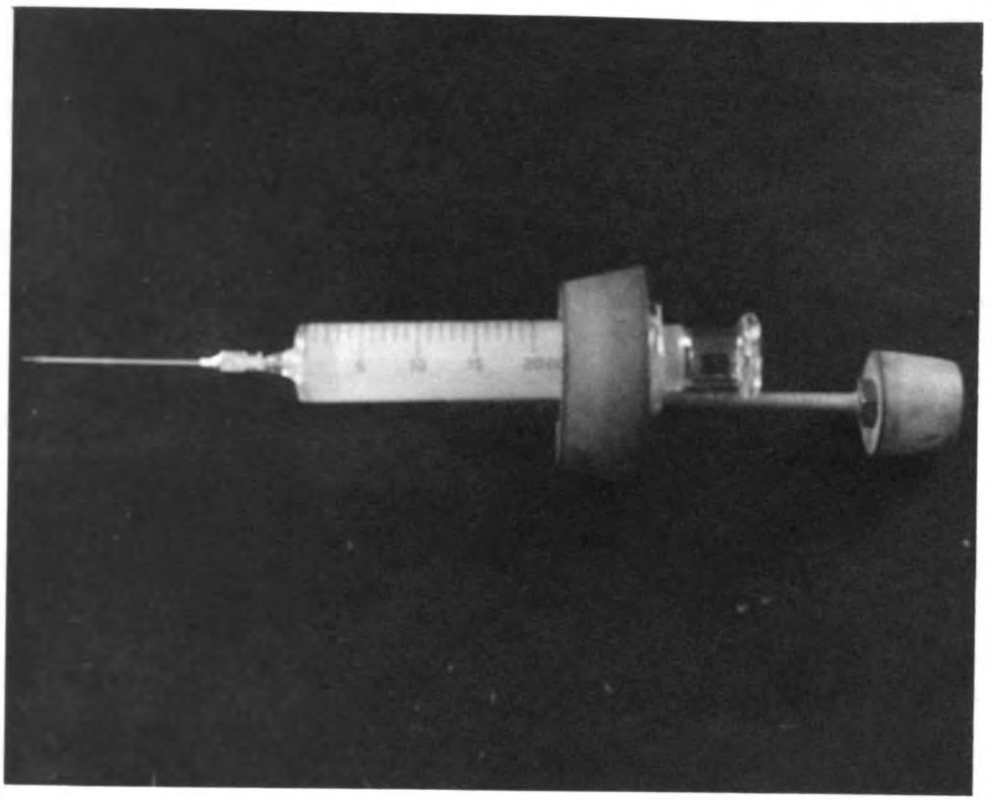

Figure III Automatic syringe apparatus used to quickly and accurately measure liquid media and agar.

measurement taken to the nearest millimeter. In cases where colony growth was irregular, measurements were made along the long and short diameter and the average taken.

b. Growth Tube Method

The growth tubes consisted of horizontal pyrex glass tubes of ½" bore, 15 inches long, bent up 2¼ inches from each end at a 60° angle. The tubes were held with their ends upright by grooved wooden supports (Fig.4).

Using the automatic syringe, six ml of 3% Difco agar was placed in each tube, the tubes plugged, placed in the supports and autoclaved.

After sterilization, six ml of sterile strawberry juice was aseptically pipetted into each of the tubes and then each end of the tube alternately raised in a rocking motion to blend the agar and juice.

Inoculation of the growth tubes was by means of a small cube cut from a PDA culture of Botrytis cinerea. The tubes were incubated at 20°C and growth measurements made periodically.

3. Liquid Media

a. Shake Culture Method

The method used for preparing the inoculum for this test was a modification of that described by Wiken, Keller, Schelling and Stockli (15).

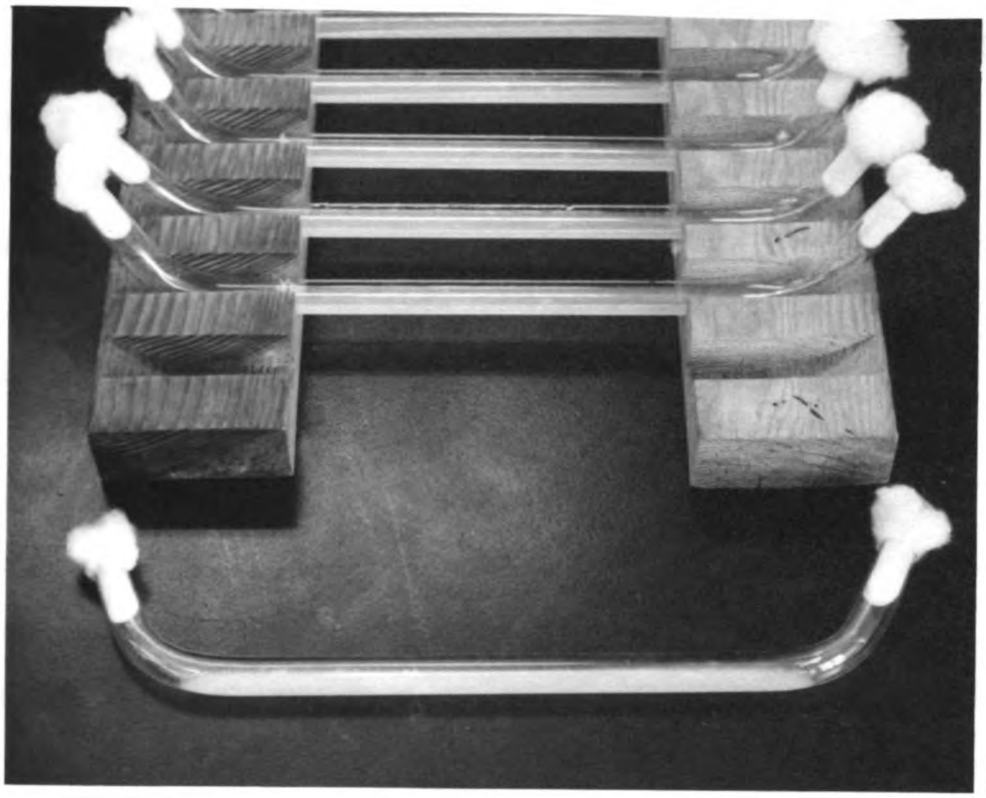

Figure IV Pyrex growth tubes showing their linear shape and method of support.

A 10 to 15 day old plate culture of Botrytis cinerea on PDA was added to a sterilized 1000 ml suction flask containing about an inch of #6 solid glass beads and enough buffer solution, pH 6, to just cover the beads (Fig.5).

The flask was swirled until the mycelium was evenly broken up into a fine suspension. One ml was aseptically pipetted into 250 ml flasks containing fifty ml of sterile strawberry juice.

The inoculated flasks were placed on a shaker table for a period of 10 days. The length of the incubation period is greatly dependent upon the fineness and density of the mycelial suspension. For comparison between runs it would be necessary to run a standard flask of potato broth or nutrient.

After 10 days incubation, the culture was filtered through a piece of parachute silk in a buchner funnel. The mycelium was scraped from the silk and placed in a pre-weighed aluminum dish and dried at 60°C to constant weight.

b. Spore Germination Method- Greenhouse Grown Berries

Spore germination tests were made in fresh juice from ripe strawberries taken at random from breeding stock growing in the greenhouse. Juice was expressed by squeezing the hulled berries on a 6 x 6 inch piece of paper toweling placed over a 7 x 7 inch piece of cheese cloth. The corners of the cheese cloth were

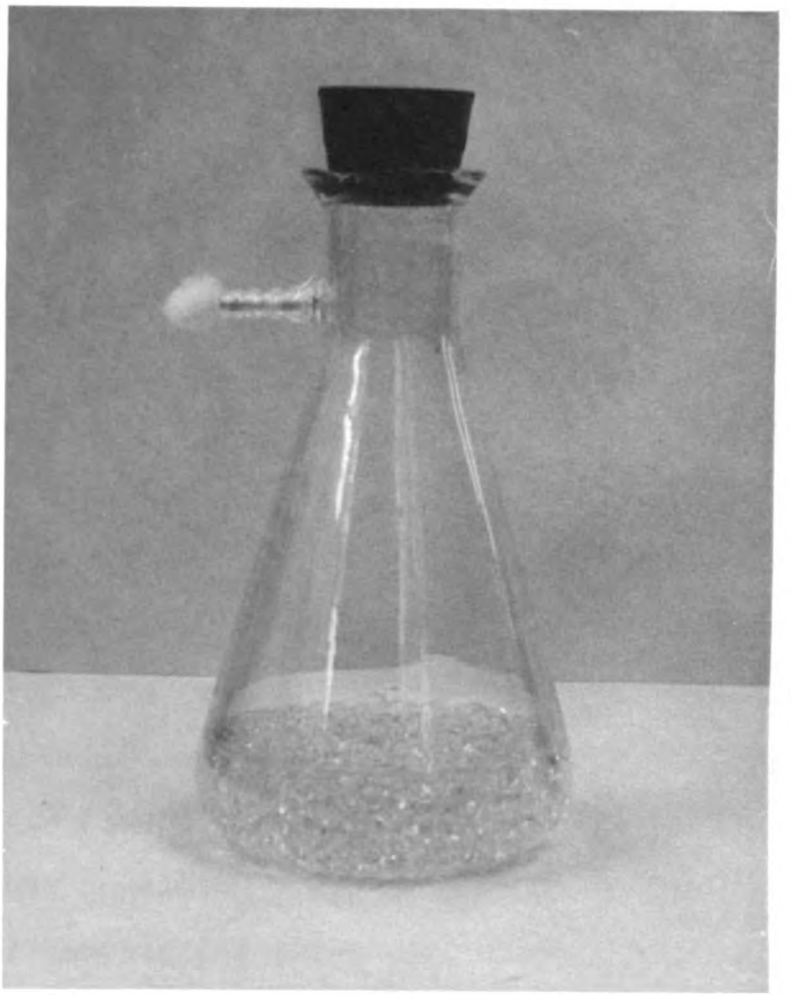

Figure V Shake culture inoculum flask, showing the #6 solid glass beads immersed in the buffer solution.

taken up to form a bag and the bag twisted to express juice from the berries. A small drop of juice was expressed onto the center of a 22 mm square cover glass and dusted with Botrytis spores. The spores were stirred into the drop with the tip of an inoculating needle. The cover glass was then inverted onto a depression slide previously prepared with a thin layer of vaseline around the edge of the depression. Observations were made after six and nine hours incubation at room temperature. After twelve hours incubation, germ tube elongation was too extensive for proper evaluation. The germ tubes of germinated spores were measured at random in units of spore length. For example, a germ tube that was relatively three times longer than its spore was recorded as three. Ten germ tubes were measured at random in each replicate.

Where possible, a series was run using both two and four berries per juice expression with four slides from each expression.

c. Spore Germination Method- Field Grown Berries

Three berries were used per variety and each stage of ripeness. Four slides were prepared for each group and measurements made on three. Ten germ tubes were measured in each slide using a micrometer eyepiece and low power objective.

4. Whole Fruit Inoculation Method

Twenty-five fruit of each variety were tested in the green, white and ripe stages of maturity. Each berry was inoculated on the side with an inverted 3 x 3 mm mycelial cube cut from a 6 to 8 day PDA culture of Botrytis cinerea (Fig.6). Mycelial cubes were used as inoculum in preference to spore inoculations because of the ease of localizing the inoculation and in locating the point of inoculation at the time of evaluation.

The inoculated berries were placed on a 13 x 17" glass plate resting on six small corks in a 14 x 18" flat. The flat was placed in a 65°F moist chamber and covered with a plastic covered screen to prevent the mist spray from contacting the berries. A temperature of 65°F is considered within the optimum range for growth of Botrytis while tending to inhibit the growth of Rhizopus contaminents.

After a four day incubation period evaluations of infection were made by cutting the berry longitudinally through the point of inoculation and measuring the depth of visible fungal development. This penetration was visible as a darkened area (Fig.6) and was measured to its deepest point and grouped in classes of 5 mm each, from 0 to 25.

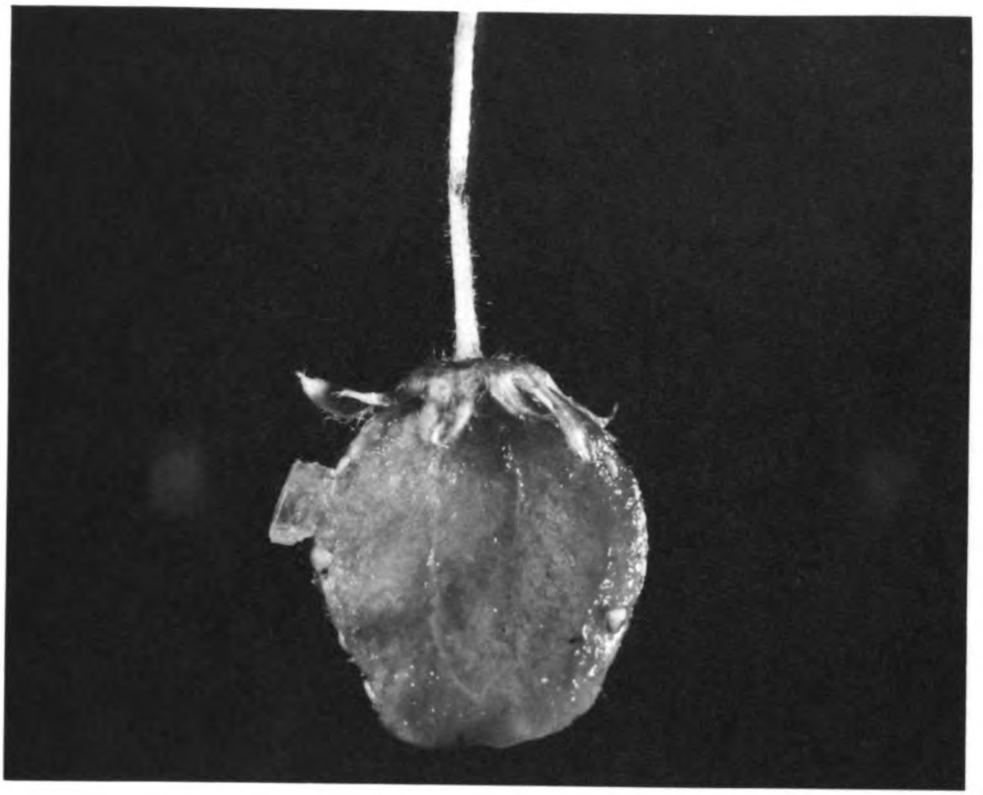

Figure VI Cross section of inoculated whole fruit after three days incubation. Area of mycelial penetration is defined by the brown area on the left side of the berry.

RESULTS

1. The pH and Per Cent Soluble Solids of the Strawberry Juices

The pH and per cent soluble solids of the various juices were measured following sterilization by Seitz filtration. This data is summarized in Table I.

As indicated in Table I the pH varied from 2.6 in selection Z-2 to 3.6 in the Albritton variety. The percentage of soluble solids ranged from 3.2 in the Earlidawn variety to 5.6 in the Albritton variety. Examination of the table revealed no dependent relation between pH and the per cent soluble solids.

TABLE I

THE PH* AND SOLUBLE SOLIDS** OF STRAWBERRY JUICE EXTRACTS

	Variety	pH	% Soluble Solids
1.	Z-2	2.6	4.8
2.	Empire	2.7	4.3
3.	South Haven 195	2.7	4.2
4.	Z-1	2.8	4.7
5.	South Haven 44	2.9	3.8
6.	Earlidawn	3.0	3.2
7.	Sparkle	3.1	3.3
8.	Red Crop	3.1	4.3
9.	Robinson	3.2	4.7
10.	Fairland	3.2	3.3
11.	Premier (Howard 17)	3.2	4.2
12.	Albritton	3.6	5.6

* Beckman glass electrode pH meter Model H-2

** Bausch and Lomb hand model refractometer

2. Petri Plate, Growth Tube and Shake Culture

Table II contains a summary of data obtained on the effect of strawberry juice incorporated in solid and liquid media in petri plates, growth tubes and shake cultures upon the growth of Botrytis cinerea. This permits a comparison of results obtained by the three methods. The rate of growth of Botrytis cinerea on Albritton using the petri plate method, is twice that which occurred on Premier (Howard 17), nearly three times that of Robinson and four times the rate that occurred on the Z-2 selections.

The order of results obtained by the growth tube method are similar to those obtained by the petri plate method as shown in the same table. The Robinson and Earlidawn varieties indicated good growth while the Empire variety failed to support B. cinerea. On the varieties Fairland, Empire and South Haven 195 where no growth occurred, inoculations were repeated several times, each failing to grow.

In the shake culture test, juice from Albritton again was most favorable for growth of Botrytis cinerea, followed in order by Premier (Howard 17), Robinson and Earlidawn, which is comparable to the results obtained in the Petri Plate method. Furthermore, these results in this test substantiate those obtained for Robinson and Earlidawn in growth tubes. Also, Z-1, Empire,

TABLE II

THE EFFECT OF STRAWBERRY JUICE IN SOLID AND LIQUID MEDIA INCORPORATED IN PETRI PLATES, GROWTH TUBES AND SHAKE CULTURE ON THE GROWTH OF BOTRYTIS CINEREA

	Variety	Average Daily Increase in Growth: Petri Plate Diameter mm	Growth Tube Length mm	Shake Culture wgt. gms.
1.	Albritton	35.0	-*	.46
2.	Premier (Howard 17)	15.6	-	.35
3.	Robinson	12.6	9.6	.13
4.	Earlidawn	12.0	8.6	.08
5.	Red Crop	12.0	-	0
6.	Fairland	9.8	0	0
7.	Z-2	8.0	-	0
8.	Z-1	0	-	0
9.	Empire	0	0	0
10.	South Haven 44	0	-	0
11.	South Haven 195	-	0	0
12.	Sparkle	-	6.6	0

* Variety not tested

South Haven 44 and South Haven 195 are similar between methods in that each failed to support B. cinerea in either method. However, there is a discrepancy, in that Red Crop, Fairland and Z-2, supported Botrytis cinerea in the petri plate method while Sparkle supported Botrytis in only the growth tube method. In the shake culture test, all three varieties, Red Crop, Fairland and Z-2, failed to support the growth of Botrytis cinerea. This may be due to a difference in the sensitivity of the two methods or it could possibly be due to the dilution of the juices by the agar when using the plate and growth tube methods.

3. Spore Germination

a. Greenhouse Grown Berries

The results of spore germination studies on juice of greenhouse grown berries is presented in Table III. This method consisted of inoculations of a hanging drop preparation of strawberry juice with B. cinerea spores. Germ tube lengths are in units of spore length.

As given in Table III, juice from selection South Haven 458 is most favorable for germ tube growth followed by South Haven 194, Earlidawn, South Haven 295 and East Lansing 1065. There is no significant difference between a two or a four berry sample. The results obtained are due to varietal influence rather than sample size.

TABLE III

SPORE GERMINATION OF *BOTRYTIS CINEREA* IN STRAWBERRY JUICES EXTRACTED FROM GREENHOUSE GROWN BERRIES

Selection	No. of Berries	No. of Reps.	Relative Germ Tube Length* Incubation Period 6 Hrs.	9 Hrs.
1. Check (Glass Dist. H_2O)	-	4	0	0
2. E. L. 1065	2	4	1.8	4.2
3. S. H. 295	2	4	2.3	6.4
295	4	4	1.8	5.8
4. Berlidown	1	4	5.5	10.2
5. S. H. 194	2	4	-	9.4
194	4	1	6.4	10.5
6. S. H. 458	2	4	-	11.4
458	4	3	6.8	11.1

* Relative length of germ tube in units of spore length

Selection L.S.D. .05 = 3.2

Sel. x Inc. Period L.S.D. .05 = 2.3; .01 = 4.1

With exception of Earlidawn, the varieties tested in this method are not included in the methods in Table II, therefore, a true correlation cannot be made between the two tables. However, Earlidawn remains favorable for the growth of Botrytis in the spore germination method as in the previous methods (Table II).

b. Field Grown Berries

The influence of variety and stage of ripeness upon the growth of germ tubes of B. cinerea were studied in this test.

As given in Table IV, five of the eight selections under test were more favorable for germ tube growth in the white stage of berry maturity than in the ripe stage. Varietal differences were also evidenced. Albritton was the most favorable for germ tube development, and South Haven 195, the least favorable.

Comparing with Table III, selections South Haven 295 and East Lansing 1065 maintained similar ranks in Table IV as in Table III, while selection South Haven 194 has shifted from the more unfavorable in Table III to the more favorable for growth in Table IV.

4. Growth of Isolates of Botrytis cinerea into Whole Berries

A study was made using whole strawberry fruit as the substrate for Botrytis cinerea in order to determine if the varietal relationships found in the juice

TABLE IV

GERMINATION OF BOTRYTIS CINEREA SPORES AFTER SIX HOURS INCUBATION IN JUICE OF DIFFERENT VARIETIES OF FIELD GROWN STRAWBERRIES AT WHITE AND RIPE STAGES OF BERRY MATURITY

Variety	Germ Tube Length* (mean) White	Ripe	Average
1. S. H. 195	11.4	9.4	10.1
2. S. H. 194	12.2	11.7	12.0
3. Premier (Howard 17)	12.1	15.4	14.1
4. Fairland	15.3	13.6	14.5
5. Robinson	19.6	14.3	16.9
6. E. L. 1065	21.6	14.4	18.0
7. S. H. 295	16.8	19.2	18.0
8. Albritton	19.5	21.0	20.0
Average	16.0	14.9	

* Average of 30 spores in units of eyepiece micrometer, low power objective.

Varieties = L.S.D. .05 = 1.3; .01 = 1.7

Maturities = L.S.D. .05 = 0.5; .01 = 2.4

V x M (groups) = L.S.D. .05 = 1.8; .01 = 2.4

preparations could also be noted in the whole berry, and to determine if these relationships exist at the various stages in the development of the fruit.

As indicated in Table V, degree of penetration of the whole fruit increased with increased maturity with the exception of selection East Lansing 1065. However, the degree of increase in penetration was dependent upon variety. For example, Premier (Howard 17) had an average penetration in the green and ripe stages of 9.1 and 16.5 mm respectively. A difference of 7.4 mm. Whereas, selection South Haven 295 had an average penetration in the green and ripe stages of 8.3 and 22.5 mm respectively, a difference of 14.2 mm. This dependence of penetration upon variety is further evidenced by the significant interaction between variety and ripeness.

The difference between varieties and between stages of ripeness and the interaction between them were highly significant. It is suggested that the variety reaction to Botrytis cinerea infection depends upon stage of maturity as well as variety and one cannot predict a reaction without taking both factors into consideration.

Correlation coefficients were calculated with data obtained in the spore germination studies on field grown berries (Table V) and penetration studies of whole fruits (Table V) as well as for different stages of ripeness within each group. Correlation coefficients are summarized

TABLE V

PENETRATION OF WHOLE STRAWBERRY FRUIT AT DIFFERENT STAGES OF MATURITY BY *BOTRYTIS CINEREA**

Selection	Stage of Fruit Development (means) Green	White	Ripe	Average
1. E. L. 1065	4.0	9.5	1.2	4.9
2. S. H. 195	4.0	6.2	7.9	6.0
3. S. H. 194	5.9	8.5	14.5	9.6
4. Premier (Howard 17)	9.1	11.8	16.5	12.4
5. Robinson	10.5	10.0	16.9	12.4
6. Fairland	10.8	10.5	17.5	12.9
7. S. H. 295	8.3	16.1	22.5	15.7
Average	7.5	10.4	13.9	10.6

* Measurement in mm.

Selection - L.S.D. .05 = 1.2; .01 = 1.6

Maturity - L.S.D. .05 = 0.8; .01 = 1.0

V x M (group) - L.S.D. .05 = 2.1; .01 = 2.7

in Table VI.

The reaction of selection East Lansing 1065 to mycelial penetration in ripe fruit was not in line with its reaction in other stages or other tests. This is particularly evident by the negative correlation coefficient obtained between the length of germ tubes in juice of white berries and the mycelial penetration of whole ripe fruit. The cause is unknown and may perhaps be due to a mode of resistance peculiar to this selection. Further testing of other selections will perhaps reveal the cause or at least reveal whether other selections will follow this same pattern. For purpose of correlation between Tables IV and V, two correlation coefficients were run, including and excluding selection East Lansing 1065.

Correlation tests including selection East Lansing 1065 gave a highly significant correlation coefficient of .97 between the growth of germ tubes in juice of ripe fruit and mycelial penetration of whole white fruit. Also, mycelial penetration of whole white fruit and mycelial penetration of whole ripe fruit gave a significant correlation coefficient of .70. All other correlation coefficients obtained were non-significant.

Correlation tests excluding selection East Lansing 1065 gave a highly significant correlation coefficient of .94 between germ tube growth in juice of ripe fruit

TABLE VI

TESTS OF CORRELATION WITHIN AND BETWEEN GERM TUBE STUDIES (TABLE IV) AND WHOLE BERRY PENETRATION STUDIES (TABLE V)

Method	Correlation Coefficient	
	A	B
Germ Tube White vs Penetration White	.46	.26
Germ Tube Ripe vs Penetration Ripe	.94**	.57
Germ Tube Ripe vs Penetration White	.99**	.97**
Germ Tube White vs Penetration Ripe	.63	-.15
Germ Tube White vs Germ Tube Ripe	.54	.46
Penetration White vs Penetration Ripe	.99**	.70*
Penetration Green vs Penetration Ripe	.69	.30
Penetration Green vs Penetration White	.52	.50

A. Selections - South Haven 195, 194, 295, Fairland, Premier (Howard 17) and Robinson.

B. Selections - East Lansing 1065, South Haven 195, 194, 295, Fairland, Premier (Howard 17) and Robinson.

** .01 level of significance

* .05 level of significance

and mycelial penetration of whole ripe fruit. Highly significant correlation coefficients of .99 were obtained between germ tube growth in juice of ripe fruit and mycelial penetration of whole ripe fruit as well as mycelial penetration of whole white fruit and mycelial penetration of whole ripe fruit. All other correlation coefficients obtained were non-significant.

DISCUSSION

Differences in the ability of the extracted juice of various strawberry varieties to support Botrytis cinerea were apparent on agar and liquid media, and by spore germination in juice.

The petri plate method indicated that juice of the varieties Albritton, Premier (Howard 17) and Robinson were highly favorable for the growth of Botrytis cinerea. The entire plates were covered within six days, whereas preparations of Fairland and Z-2 varieties had not shown any increase until the fourth or fifth days respectively and no growth was visible at all on the Empire, South Haven 44 and the Z-1 preparations.

The order of results obtained in the growth tube method were similar to those obtained by the petri plate method with the exception of the Fairland variety which displayed growth in the petri plate but failed to support Botrytis cinerea in this method.

The results in liquid media for Albritton, Premier (Howard 17), Robinson and Earlidawn were similar to those obtained in solid media. However, Red Crop, Fairland and Z-2 varieties which supported Botrytis cinerea in the petri plate and growth tube methods failed to support it in the shake culture method. This could be attributed to a difference due to dilution of the juices by the agar in the petri plate and growth tube methods.

Comparison of pH and percent soluble solids of the juices with the growth responses obtained in the petri plate, growth tube and shake culture methods of evaluation, indicated with the exception of selection Z-2, which supported Botrytis cinerea in the petri plate method only, all varieties having a pH below 3.0 consistently failed to support Botrytis cinerea in all three methods. No correlation was indicated between results obtained and percent soluble solids, or varieties having a pH above 3.0.

Results obtained in the spore germination method with juice from greenhouse grown fruit disclosed that this method requires a minimum number of fruit. Results of varieties in common between this method and previous methods were consistent.

In the spore germination study of field grown berries, an attempt was made to determine the influence of maturity or stage of ripeness, and variety upon the growth of the germ tubes of B. cinerea. The results indicate that the variety as well as stage of ripeness are determining factors of response.

Studies on whole fruit response to Botrytis inoculation indicate that the penetration of the causal organism is dependent upon the variety and also upon the stage of maturity and that one cannot predict the effect of one without the other.

Tests of correlation were run on data obtained in spore germination studies of field grown berries and mycelial penetration studies of whole fruit as well as different stages of ripeness within each group.

The non-significant correlation between the length of germ tubes in inoculated juice of white berries with lengths obtained in juices of ripe berries suggests that the reactions due to maturity are dependent upon variety. There is however, highly significant correlation between mycelial penetration of whole white berries and mycelial penetration of whole ripe berries. One underlying factor which may explain this apparent contrast is that in the duration of the test (three days) the berry is increasing in maturity and at the end of the test the difference between the ripe and the white fruit may be negligible. For this same reason the lack of correlation between the length of germ tubes in juice of white berries and mycelial penetration of white berries may be explained.

The most significant correlation biologically as well as statistically is that between the germ tube length in the juice of ripe fruit and the mycelial penetration of whole ripe fruit. This suggests that the spore germination method may be used as a preliminary screening in a breeding program followed by whole fruit penetration studies later in the program. Additional

testing in subsequent seasons should disclose whether a definite pattern of response exists.

SUMMARY

An investigation of techniques that could be employed in a laboratory screening test to evaluate relative varietal fruit susceptibility to Grey Mold (Botrytis cinerea) was undertaken.

Strawberry juice was extracted and sterilized by Seitz filtration. These strawberry juices were incorporated in both liquid and solid media and evaluated for growth of Botrytis cinerea. Growth responses were consistent for certain strawberry varieties throughout these methods.

Comparing pH and percent soluble solids of the juices with the growth responses obtained in the petri plate, growth tube and shake culture methods of evaluation indicated with the exception of L-2, which supported Botrytis under the petri plate method only, all varieties having a pH below 3.0 consistently failed to support Botrytis in all three methods. No correlation was indicated between results obtained and percent soluble solids, or varieties having a pH above 3.0.

Studies of spore germination using hanging drop preparations of juice directly extracted from berries and inoculated with Botrytis spores was undertaken. An attempt was made to determine the influence of maturity or stage of ripeness of the fruit and variety upon the growth of the germ tubes of B. cinerea.

Tests of correlation between results obtained in the germ tube length in the juice of ripe fruit and the mycelial penetration of whole ripe fruit gave a highly significant correlation coefficient. It is suggested that the spore germination method be used as a preliminary screening test in a breeding program followed by a whole fruit penetration studies later in the program. Additional testing in subsequent seasons should disclose whether a definite pattern of response exists.

BIBLIOGRAPHY

1. Anderson, H. W. 1946. Strawberry fruit rots and their control. Trans. Ill. Hort. Soc. 80: 239-243.

2. Anderson, J. P. 1924. Botrytis cinerea in Alaska. Phyto. 14: 152-155.

3. Bain, D. C. 1944. Strawberry diseases in Louisiana. Pl. Dis. Rep. 28: 324.

4. Blackman, V. H. and E. J. Welsford. 1916. Studies in the physiology of parasitism II infection by Botrytis cinerea. Ann. of Bot. 30: 389-398.

5. Brown, W. 1916. Studies in the physiology of parasitism III on the relation between 'infection drop' and the underlying host tissue. Ann. of Bot. 30: 399-406.

6. Fulton, R. H. 1955-1958. Unpublished data.

7. Miller, P. M. and P. E. Waggoner. 1957. Dispersal of spores of Botrytis cinerea among strawberries. Phyto. 47: 24-25 (abst.).

8. Stevens, F. L. 1914. A destructive strawberry disease. Science 39: 949-950.

9. Stevens, N. E. 1916. Pathological histology of strawberries affected by species of Botrytis and Rhizopus. U.S.D.A. Jour. Ag. Res. 6: 361-366.

10. _____. 1922. Rots of early strawberries in Florida and Southern California. Amer. Jour. Bot. 9: 204-211.

11. _____. and R. B. Wilcox. 1918. Further studies of the rots of strawberry fruits. U.S.D.A. Bul. 686.

12. Stoddard, E. M. and P. M. Miller. 1956. Control of Grey Mold on strawberries under greenhouse conditions. Fl. Dis. Rep. 40: 443-445.

13. Tribe, H. T. 1955. Studies in the physiology of parasitism XIX on the killing of plant cells by enzymes from Botrytis cinerea and Bacterium aroideae. Ann. of Bot., N.S. 30: 351-368.

14. Webb, R. W. 1921. Studies in the physiology of the fungi XV. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Ann. Mo. Botan. Garden 8: 283-341.

15. Wiken, T., H. G. Keller, C. L. Schelling and A. Stockli. 1951. Uber die verwendung von myzelsuspensionen als impfmaterial in wachstumsversuchen mit pilzen. Experientia 7: 237.

16. Wilson, D. J. 1954. Cultural experiments with strawberries. Rep. East Malling Res. Sta. 149-151.

17. Wormald, H. and R. V. Harris. 1937. Notes on plant diseases in 1937. Rep. East Malling Res. Sta. 181-186.